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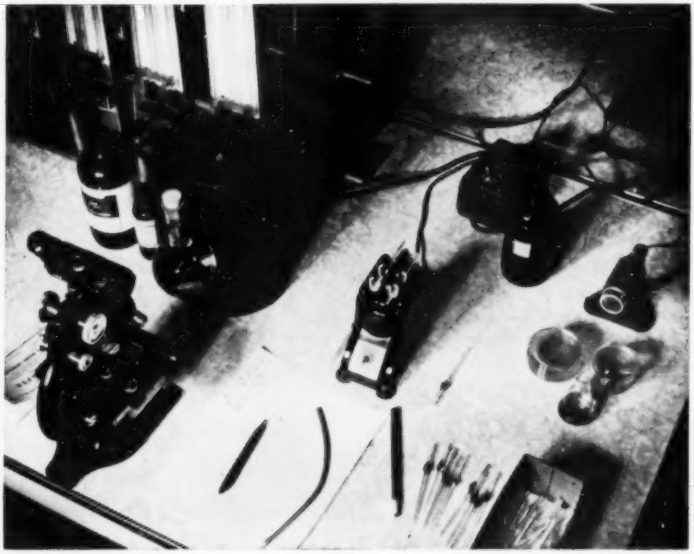

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EDITORIAL

COINCIDENCE OR BY DESIGN?

THE action by the Antitrust Division of the Department of Justice against the Northern California Pharmaceutical Association may well be just one part of a carefully designed plan to discredit the entire public health team in the United States. Those who watched the Senate Antitrust and Monopoly Subcommittee in action against the drug industry last year could not help but writhe at the studied efforts used to convince the American public that these were robber barons greedily seeking to exploit the sick and the poor.

It will be interesting to see how the medical profession fares, for it is next on the government timetable. It seems certain that it will be "exposed" as callously ignoring the needs of the aged and infirm in not supporting socialized medicine for all over 65.

In exploring the motives behind these attacks on pharmacists, physicians, and the drug industry, one has the lurking suspicion that it is the long-range hope of some in government circles to see the entire medical care team completely socialized and operated by the Department of Health, Education, and Welfare. To do this first requires convincing the public that our present system is not only inadequate but operated by scoundrels who are guilty of collusion to better exploit the suffering patient. It could, of course, be pure coincidence that these attacks on several fronts have occurred so close together and so well timed but there seems a greater likelihood that some able minds are at work on this problem—minds which, we may add, are highly skilled in human psychology and the molding of public opinion. We suspect that some of these are persons who are in key advisory positions but who themselves are not often in the public eye.

It is our prediction that the public health team is fighting a last-ditch battle for survival as free and independent practitioners of the health professions or as businessmen operating under the free enterprise system. We doubt very much whether this fact has been fully appreciated by those whom it affects most. Otherwise, our ranks

would be closed and one would not hear or see the all-too-frequent signs of internecine disputes which weaken our defense and make us ineffective. Two things are certain—we are not struggling against adversaries who are unskilled, ineffective, or complacent, and, viewing the battle objectively, there is surely reason for pessimism on the part of those who believe in our present system.

Seen in the light of our ultimate national destiny, one cannot help but see the signs of advancing state socialism and, indeed, even communism springing up all about us. Is this indeed the only ultimate solution of our social and national problems? If it is, then we should accept it. If on the other hand there are still those who believe in human dignity, individual initiative, and freedom of thought and action, then they had better engage their adversaries without delay. Time is short and those who believe in freedom have not been doing very well.

L. F. Tice

TREATMENT AND PROPHYLAXIS OF RADIATION DAMAGE

By Philip Needleman *

Effects of Radiation

EXPOSURE to ionizing radiation manifests itself in different types of injuries, depending upon: the dose, tissue area irradiated, duration of exposure, as well as intensity and wave length of the source (1).

The biological effects of ionizing radiation lead to an alteration of tissue constituents, either directly by interaction with target molecules or indirectly by formation of active, free radicals from tissue water molecules (2).

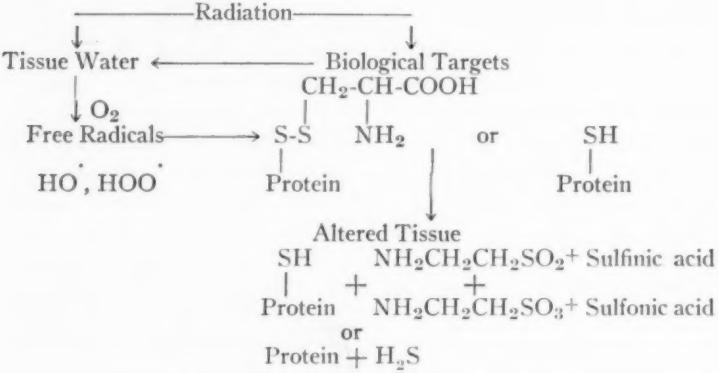

FIGURE 1. BIOLOGICAL EFFECTS OF RADIATION.

The primary effect of radiation is on body water, which is decomposed into hydrogen and hydroxyl ions (4). These ions combine with the oxygen in the body to form the highly reactive oxidizers H_2O_2 and HO_2 (1, 5). Disulfides, as well as sulfhydryl—containing essential enzymes, are exceedingly sensitive to the oxidizing effects of free radicals. The sulfur groups are mainly converted to the oxidation products, sulfinic and sulfonic acids, and are also partly split off

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as hydrogen sulfide. There is a differential susceptibility of organic compounds to irradiation which may manifest itself in many ways. A few examples are: deamination and decarboxylation of amino acids, hydroxylation of benzoid structures, formation of unsaturated fatty acid peroxides, polymerizations (2).

The premise of drug protection against radiation is that the agent is present in the system prior to irradiation and that it will act to reduce or prevent damage (3). This protection may be achieved by one of or any combination of the following mechanisms (6):

A. Trapping the free radicals arising from body water upon irradiation.

B. Increasing the resistance to radiation by combining with sensitive biological molecules.

C. Reducing the radical yield achieved by decreasing the oxygen tension in the tissue.

D. Stimulating the body's repair processes.

Interest in prophylaxis and therapy has a comparatively brief but active history. The initial studies were reported by Patt and coworkers (7) about a dozen years ago. Since that time, the list of chemical agents that manifest a protective effect against some phase of radiation is impressive (8). The purpose of this review is to consider those agents that are most effective and promising as well as those that offer insight into new pathways of research in this field.

Sulfur-Containing Agents

The protective agents, which have been proven most effective, are those that possess a sulfhydryl group. Experimentation with these agents first began after it was reported that the amino acid, cysteine, reduced the number of deaths in irradiated experimental animals (3).

Considerable interest is focused on the mechanism of action by which sulfhydryl-containing compounds achieve their effectiveness. Three theories of the mechanism of protection exhibited by sulfhydryl compounds are:

A. Protection by inactivation of free radicals since a number of sulfhydryl-containing agents react rapidly with free radicals (2).

B. Protection by removal of free radicals before they are able to react with body proteins, thereby protecting the cellular target molecules (*2*, *9*).

C. Protection by formation of mixed disulfides; a series of SH- and SS- containing agents have now been shown to be effective as protective agents. These agents become attached *in vivo* to tissue constituents through the formation of mixed disulfides. There is great correlation between the protective effects of thiols and disulfides and their ability to form mixed disulfides with biological target groups. Studies of the mixed disulfide mechanism has led to the following hypotheses (*2*):

1. Protective compounds of the sulfhydryl group form mixed disulfides in the body with the SS- and SH- groups of tissue proteins, thus giving temporary protection. The mixed disulfide is eventually reduced by gluathione reductase and, when this occurs, the proteins are once again subject to the effects of radiation (*2*).

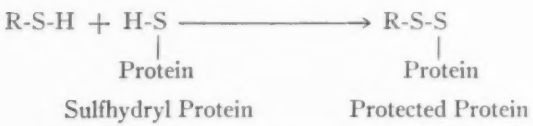


FIGURE 2. MIXED DISULFIDE FORMATION.

2. Mixed disulfide formation reduces the chance of irreversible alteration of target molecules by the indirect effects of radiation; that is, by free radicals. The free radicals attack a disulfide group, oxidizing one of the sulfur atoms to a sulfinic or sulfonic acid group; the other sulfur is reduced to an SH- group (Figure 1). Protective agents modify the target SS- and SH- groups by mixed disulfide formation. Since a radical may attack either one or the other of the two sulfur atoms, it can be assumed that, in 50 per cent of such cases, the protective agent, sulfur, is been protected (*2*).

3. Mixed disulfide formation also serves to dissipate the energy of radiation in a non-injurious manner. Direct radiation again acts to rupture the mixed disulfide bond and

results in the formation of one SH- group and a sulfinic or sulfonic acid group (Figure 1). Thus, once again, the chemical combination of the protective agent with the target molecule represents a 50 per cent protective action of body proteins (2).

Mercaptoalkylamines

The discovery that cysteine offers protection against radiation spurred examination of both amine and sulfhydryl compounds. This search led to the discovery of 2-mercaptoethylamine (MEA), also termed cysteamine, which is produced by the decarboxylation of cysteine (10).

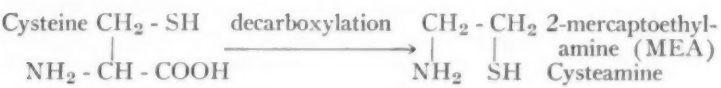


FIGURE 3. MEA FORMATION.

Cysteamine has been shown to be highly effective in protecting experimental animals. In fact, it is more easily and more cheaply synthesized than cysteine. An additional advantage is that it is about five times as effective as cysteine in equimolecular quantities. However, cysteamine has a greater toxicity than cysteine (12).

The amino acid cystine was found to offer ineffective protection; whereas, cystamine was noted to increase the survival time of mice. This effect was investigated further. As a result, cystamine was found to cause a rapid formation of histamine in the body. Histamine and other amines exert their protective effects by altering tissue oxygen tension. No such histamine release is noted with cysteamine (13).

The metabolisms of cysteamine and cystamine were investigated in mice using S^{35} labeled compounds. These agents were found primarily in the blood, bound to the protein constituents through the mixed disulfide formation. There was only a minute quantity of blood radioactivity present as free cysteamine or cystamine. Therefore, interest was focused on the interaction of these agents with biological target areas and their ability to protect against radiation (14).

A consideration of the effects of structural changes on the protective activity of nitrogen and sulfur-containing compounds serves as a useful tool in understanding these agents:

A. Sulfur must be present in the molecule either in the form of a free sulfhydryl group or a disulfide group. Substitution of the sulfur group as in the S-alkyl compounds (thioethers) causes loss of protective effectiveness. Although some radioprotective compounds of this class seem to be exceptions to the rule, having substituted S groups, this is only an apparent exception because they undergo a rapid intramolecular rearrangement *in vivo* to give a free SH- group. Two such agents are alphahomocysteine thiolactione and S-acetylcysteamine (*2*).

B. Amine groups are essential for activity, and substitution in the amine group greatly affects protective ability. Acylation and arylation of the nitrogen abolish protective effectiveness; whereas, alkylation with aliphatic or alicyclic compounds cause a slight increase. However, the latter increase in effectiveness is accompanied by an increase in toxicity. The guanidyl group seems to add considerably to the activity as compared to the amine group. Both 2-mercaptoethylguanidine and 3-mercaptopropylguanidine are more active than cysteamine as radiation protective agents in experimental animals (*2*).

C. Carbon chain length also influences protective ability. The most effective straight chain compound contains two or three carbon atoms. Increasing the carbon chain of 2-mercaptothylamine (MEA) by one carbon to give 3-mercaptopropylamine (MPA) makes the compound twice as active; whereas, further separation of the amine and the sulfhydryl group as in 4-mercaptobutylamine (MBA) reduces protective activity to one-fifth that of MEA (10).

D. Protective effectiveness is decreased by the presence of a carboxyl group in addition to the amine and, on a molar basis, cysteine is five times less active than cysteamine (*2*).

The most effective member of this group is a combination of a basic and an SH group separated by not more than three carbon atoms. The protective ability of this group may be related to its ability to form, during irradiation, a "resonance stabilized free radical" (Figure 4), which would be capable of preventing further free radical reactions with biological material (10).

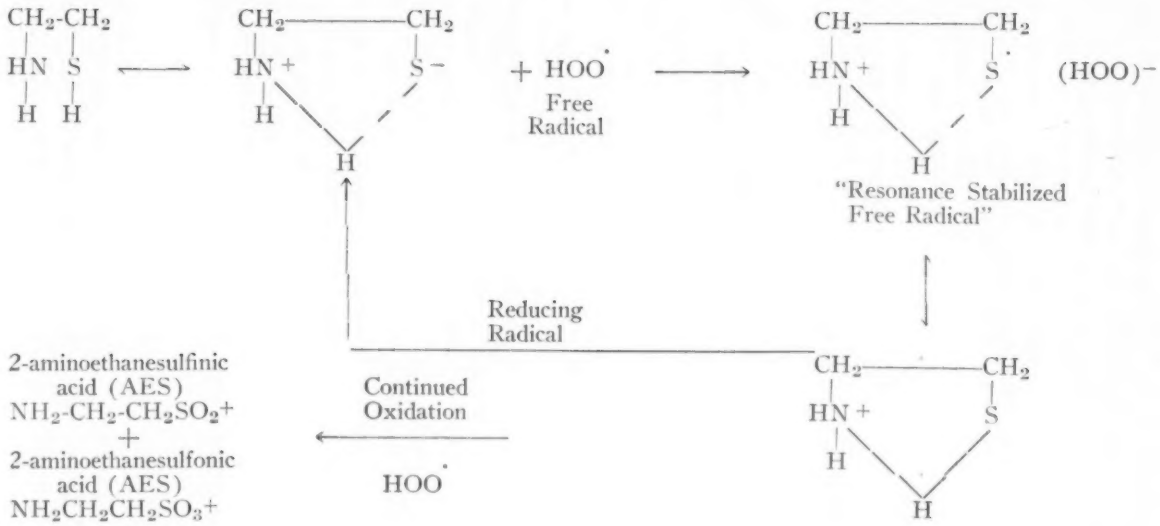


FIGURE 4. FORMATION OF "RESONANCE STABILIZED FREE RADICAL"

The free radical form of MEA could possibly resonate between the above forms. A representation of the free radical reactions caused by irradiation in the presence of MEA has been given by Doherty *et al.* (10) as follows:

A. $H_2O + O_2 \xrightarrow{\text{Radiation}} HOO^{\cdot} + OH^{\cdot}$ — These are the primary radicals released on irradiation of body water.

B. $MEA + HOO^{\cdot} \longrightarrow MEA^{\cdot} + H_2O_2$ — The resonance stabilized protective radical is formed.

or

$MEA + OH^{\cdot} \longrightarrow MEA^{\cdot} + H_2O$

C. $MEA^{\cdot} + OH^{\cdot} \longrightarrow MEA + O$ — A possibility is that the free radical of MEA may regenerate MEA.

D. $MEA^{\cdot} + MEA^{\cdot} \longrightarrow AED$ — The radical reacts with another molecule of itself to give 2-aminoethyldisulfide (AED).

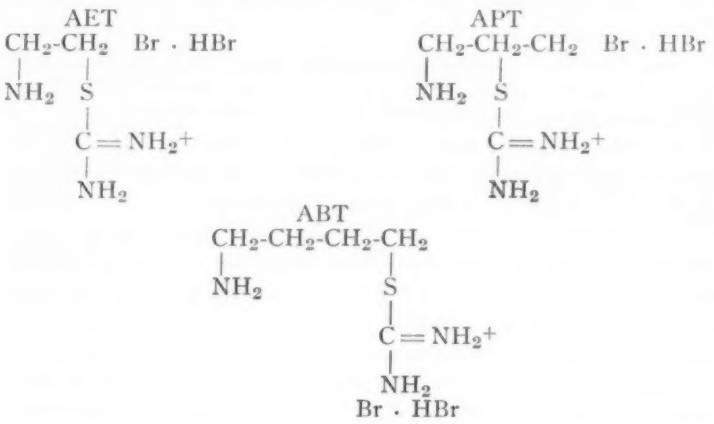
E. $MEA^{\cdot} + HOO^{\cdot} \longrightarrow AES$ — Otherwise, the radical may be further oxidized to 2-aminoethanesulfinic and 2-aminoethanesulfonic acids (AES).

This series of reactions offers a possible explanation of chemical pathways by which the mercaptoalkylamines deal with free radicals that have been produced by radiation. In support of this argument, the increased activity of 3-mercaptopropylamine is explained by the probability of resonance stabilization, which is caused by the formation of a more stable six-membered ring intermediate. In the case of 4-mercaptobutylamine, the decreased protective ability is explained on the basis of the improbability of the formation of a seven-membered ring (10).

Mercaptoguanidines

By systematic modification of the structure of cysteamine, the compound S, 2-aminoethylisothiuronium bromide hydrobromide (AET) was discovered (3, 15). The thiuronium compounds have

shown themselves capable of being effective antiradiation agents (16). AET and its homologue, S, 3-aminopropylisothiuronium bromide hydrobromide (APT), were shown to be equally effective against the lethal effects of radiation in experimental animals. S, 4-aminobutylisothiuronuim bromide hydrobromide (ABT), the next higher homologue, is ineffective as a radioprotective agent.



Thiuronium compounds, which protect against radiation, give a positive nitroprusside test for the SH- group. This is explained by the theory that AET exists in a variety of forms (15).

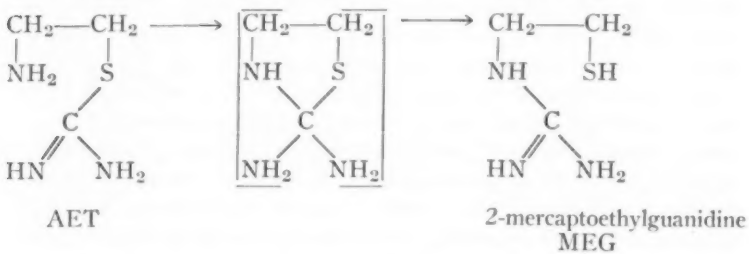


FIGURE 5. INTRATRANSGUANYLATION OF AET

This diagram (Figure 5) represents the events which occur when AET is boiled or allowed to stand; and it is these conditions which give a positive nitroprusside SH- test and exhibit protection against irradiation. AET intratransguanylates to MEG, and APT intratransguanylates to 3-mercaptopropylguanidine (MPG); whereas, ABT is a compound which cannot cyclize in this fashion to produce a free sulfhydryl group and which lacks radioprotective effectiveness (15).

AET or MEG has a lower toxicity than a molar equivalent of MEA, its parent compound, and it is twice as effective as MEA at equivalent dose levels (17, 18).

A brief consideration of the structure activity relationships of the mercaptoguanidines will help to clarify the effectiveness or lack of effectiveness of various radioprotective agents.

To prove the necessity of the presence of an amino group in the active isothiuronium compounds, Doherty prepared a series of salts, in which the amino group of AET was replaced by other functional groups such as nitrile, hydroxyl, or carboxyl. None of these compounds showed any radioprotective activity; thus, the presence of the basic group appears to be essential for protective activity. To further prove this requirement, these investigators prepared two series of compounds. In the first series, the amino group hydrogens were replaced by alkyl or substituted alkyl groups. These dialkylamino and trialkylamino compounds proved ineffective as radioprotective agents and increased the toxicity of these agents as compared to their parent compound. In the second group, the hydrogen atoms of the amino group were replaced by a ring system. These cyclic nitrogen compounds also were ineffective and toxic (17). Thus, replacement of the free amino group, or substitution of it, cannot occur without the loss of protective activity (19).

To be effective as a protective agent requires the presence of a guanido group in conjunction with a thiol group. These should be separated by two or three carbon atoms, at the most, to maintain maximum protective activity. The mercaptoguanidines that have been found to be the most effective thus far are MEG and MPG (converted from AET and APT respectively). MPG as a radioprotective is twice as active as MEG on a molar basis; however, the toxicity increases in the same ratio (17).

The conversion of the amino group to a guanido increases the protective activity and therapeutic ratio in both ethyl and propyl thiols. The guanido and thiol are brought close together by the formation of a five- or six-membered ring; and, as previously noted, only those aminoalkylisothiuronium compounds, which are capable of intratransguanylating to the five- or six-membered cyclic intermediate, will have protective activity (17).

Thiazolidines and Aminoalkylthiosulfuric Acid

Mercaptoalkylamines have, as their functional class, the grouping N-C-C-S, in which the nitrogen is basic and the sulfur is of mercaptan character. The functional molecule of alkylisothiuronium salts can be represented as: $R_1\ R_2$ N-C-C-S · C $(=NH) \cdot NH_2$ 2 · H

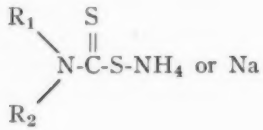
Thiazolidines, having the skeleton and aminothiosulfuric acids, $R_1R_2NC\text{-}C_3S \cdot SO_3H$ were developed as a modification of the above compounds in the hope of finding a more potent but nontoxic protecting agent.

These agents do possess a radioprotective activity equivalent to MEA, but they are more toxic in their effects. The activity of these agents is possibly attributed to their *in vivo* decomposition to the corresponding mercaptans (20).

Dithiocarbamates

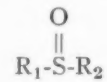
Dithiocarbamates have shown some radioprotective activity against the lethal effects of radiation (21). The most effective agents in this series are ammonium dithiocarbamates which, on a molar basis, are one-third as active as MEA. Alkylation of the amine or increasing the length of the alkylating group does not alter the protective activity but does increase the toxicity (2). The dithiocarbamate structure, itself, seems to be the functional part of the various active molecules, since substitution or addition to the molecule decreases the protective ability of these compounds (21). The mode of action, by which these agents function, has not yet been

elucidated. The general formula for this group may be represented as follows:



Sulfoxides

Sulfoxides and derivatives of dimethyl sulfoxides have been investigated for radioprotective activity against lethal doses of X-rays in mice. Only dimethyl sulfoxide exhibited a significant protective activity in that it protected 70 per cent of the experimental animals against lethal doses of X-rays. However, it does not compare in effectiveness to AET. Of the other sulfoxides tested, only five were found to possess even weak protective activity. These were: methyl ethyl sulfoxide, tetramethylene sulfoxide, d,l-methionine sulfoxide, diphenyl sulfoxide, and bis-tribromomethyl sulfoxide (22). The general formula for this group may be represented as follows:



Mercaptopyridoxine and BAL Derivatives

The compound, 5-mercaptopyridoxine, exerts a radioprotective action for mice and *E. coli;* whereas, pyridoxine is inactive. Although 4,5-dimercaptopyridoxine is ineffective in protecting mice, it still offers protection to bacteria. The protective action of both these sulfhydryl derivatives on *E. coli* is abolished when irradiation is carried out under aerobic conditions, therefore suggesting that the mechanism involves the production of anoxia (23).

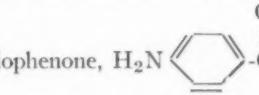
BAL is a sulfhydryl compound which has no marked radioprotective activity. However, certain Russian workers prepared a BAL derivative which was radioprotective and less toxic. In this derivative, the hydroxyl group was replaced by a sulfonate group (23).

Miscellaneous S-Containing Protective Compounds

The SH-containing amino acid, glutathione, is effective; whereas, methionine, which contains $-SCH_3$, is inactive (17).

Thioureas and various inorganic sulfur-containing compounds (such as, thiosulfate and colloidal sulfur) offer some protection in large doses.

Anoxia

Oxygen's presence alters the effects of radiation by increasing the yield and actions of free radicals (24). In addition, oxygen may also alter some enzymes and cause damage by increasing their sensitivity. In any case, anoxia-producing drugs may act to depress free radical formation (1). Methemoglobin-producing drugs, such as para-aminopropiophenone,  , given in a dosage that converts 30-40 per cent of the blood hemoglobin, decrease the oxygen tension and offer protection against radiation. Nitrite is also fairly effective, although it is toxic. Carbon monoxide produces a similar effect due to the formation of carboxyhemoglobin (11).

Radioprotective action by reduction of oxygen tension (6) is supported by the finding that the magnitude of a protection is similar for oxygen deprival and for protective agents (2).

Cyanide used in non-toxic doses offers protection to 50-80 per cent of experimental animals exposed to radiation. Malononitrile, which liberates cyanide in the body, is a good protective agent; whereas, thiocyanate and cyanate are ineffective. Cyanide inhibits cytochrome oxidase and heavy metal enzymes (catalases, peroxydase, catechol oxydase), thereby blocking oxidative phosphorylation and the utilization of oxygen by the mitrochondria (11). The cytochrome oxidase system is also inhibited by diphenylamine which increases in effectiveness when given in combination with para-aminopropiophenone (9). Formate, another inhibitor of catalase, is a radioprotective substance; whereas, acetate is ineffective both as radioprotective and as an enzyme inhibitor (11).

Epinephrine and Vasopressin offer some protection, presumably as a consequence of vasoconstriction which causes a decreased availability of oxygen (25, 26). Likewise, the protective effects of Methoxamine (27) and of serotonin creatinine sulfate probably lie in their vasoconstrictor activity, thereby producing a transient tissue anoxia similar to epinephrine (28).

It must be pointed out that the anoxia-producing agents usually have to be given in near toxic doses, thereby reducing their usefulness alone. In addition, the body can tolerate only a limited amount of anoxia. Thus, agents with increased potency can hardly lead to improved radioprotection. These agents do have their place and can greatly augment the radioprotective action when used in combination with the more specific protecting agents (9).

Chelating Agents

Various agents, which have radioprotective activity, also have the ability to form chelates. Therefore, there may be a correlation between protective ability and combinations with metals. The activity of chelating agents could be due either to the fact that heavy metals (such as copper) are involved in radiation reactions, or to the fact that the chemical characteristics, necessary to confer chelating ability are the same as those necessary for radioprotection (12).

The general structure of many of the radioprotective agents can be written as $X\text{-}CH_2\text{-}CH_2\text{-}Y$ or $X\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}Y$, where X and Y are groups capable of coordinating with metal ion. Thus, these agents are capable of forming five- or six-membered rings upon chelation. Typical examples are MEA, AET, glutathione, and diethyl dithiocarbamate (29).

Jones (29) explains the effectiveness of chelates in the following manner: Radiation causes the release of metal constituents of tissue, which then enter the circulation and are capable of inhibiting certain enzymes and co-enzymes such as ATP, carbonic anhydrase, and arginase. Protective agents chelate these metals into a stable complex and thereby prevent enzyme inhibition. An alternative explanation is one in which the radioprotective agents chelate with the hydroxylonium or hydrogen peroxide free radicals and thereby decompose them.

Other Radioprotective Agents

The hormones that have shown some effectiveness include desoxycorticosterone acetate (20, 30), and L-triiodothyronine (31). These agents may possibly act to alter the metabolism of the radiation damaged tissue.

Even though 5-hydroxytryptamine has been found to be an effective radioprotective agent, 5-hydroxytryptophan, its precursor, does not exhibit the same quantitative protection against radiation. The slight activity of the latter agent is due to the formation of the amine *in vivo* following decarboxylation of tryptophan (32).

Reserpine has also proved to be an effective protective agent in modifying radiosensitivity. It is believed to function by the endogenous release of 5-hydroxytryptamine and possibly catecholamines. Reserpine is effective in low doses and, in experimental reports, it appears to be as effective as cysteamine (2).

Ganglionic blocking agents were administered to mice prior to irradiation in order to investigate the benefits that might be derived by blocking autonomic nervous transmission. These agents exhibited no beneficial effects in radiation injury. They neither increase nor decrease survival time or mortality rates (33). Cholinergics show a slight decrease in radiation-induced mortality in mice. This radioprotective effect is abolished by atropine sulfate (34). There is a need for more experimental study of parasympathomimetics and sympathomimetic agents in this field.

Post-Irradiation Therapy

The object of post-irradiation therapy is to deal with the immediate damage and to prevent development of any delayed effects of radiation (3). The agents or procedures that offer some effectiveness include:

A. Blood transfusions, in which approximately one-fifth of the blood of an irradiated individual is replaced.

B. Transfusions of bone marrow homogenates. Transplants of bone marrow cells provide the individual with blood-forming cells until his own can regenerate. The problem that arises in any graft of foreign tissue is that the body rapidly destroys such tissue through antibody production. However, in the irradiated individual, the ability to produce antibodies has been lost; therefore, the transplanted bone marrow may survive temporarily (3).

C. Administration of antibiotics. Antibiotics are useful in post-irradiation therapy when the damages caused by the radia-

tion itself are not too severe (36). Radiation reduces or abolishes active or passive immunity to bacterial infection and animal parasite infections; also, antibody formation is greatly inhibited (37). Thus, antibiotics act to protect the irradiated individual until natural defense mechanisms are regenerated (36).

D. Administration of antihemorrhagic agents. Antihemorrhagic agents, such as Toluidene blue and Protamine, are used to counteract the incoagulability of blood which results from whole body irradiation (30).

Conclusion

Significant progress has been made in the last decade in the chemical protection against radiation. However, the agents presently being tested are still too toxic for extensive human use at high radiation dose levels. They should prove useful in furthering the understanding of the functioning of protective agents in human tissue.

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INTERFERON—AN INHIBITOR OF VIRUS MULTIPLICATION

By Louis Gershenfeld *

I. Introduction

INTERFERENCE between viruses even among those not necessarily related so that the growth and causation of disease of one is affected by the other has been known for more than two decades. Such interference was first noted between viruses causing diseases in plants. It also occurs among bacteriophages or bacterial viruses. Inactivated or attenuated viruses just as live viruses may produce interference effects. Here, however, the method used to inactivate the virus is of importance. Formaldehyde-treated virus destroys the interfering property. Ultraviolet-irradiated virus results in attenuated virus preparations which are uniformly effective in interference. Heat-treated virus (for attenuation) gives preparations which are variable in their results.

During an investigation of the virus interference phenomenon, Isaacs and Lindermann (1) observed that, when inactivated virus was incubated with chick chorio-allantoic membrane (CAM) cells in a buffered salt solution, a substance was formed in the cells and released into the fluid, which itself had the property of interferring with the growth of viruses. This substance they called "interferon." When partially purified "interferon" is added to fresh membranes, the addition of virus does not result in infection. Interferon has not been isolated from the virus particle; it is regarded as a protein material synthesized in the host cell after infection. The mechanism of its action is not definitely known. Its appearance and many of its properties and characteristics, however, have been confirmed by Henle *et al.* and Tyrrell (3, 6). For a detailed review and references on viral interference, the reader is referred to Wagner's presentation (4).

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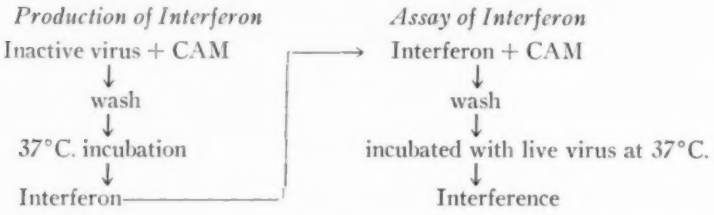
A. Schematic Diagram of Interferon Production.



The peak production of interferon occurs at 12 hours and decreases slightly at 24 hours.

II. Characteristics of Interferon

Fundamental studies by the Mill Hill group (2) indicated that interferon: (a) Was a protein that can be inactivated by proteolytic enzymes, such as trypsin or pepsin. (b) Is smaller in size than antibody molecules. (c) Can be produced by cells of different species of animals (1, 3-6). (d) Is produced by many viruses, both active and inactive. The multiplication of the second or challenge virus is inhibited intracellularly. (e) Works most efficiently in the same system of cells or animal species in which the interferon was prepared (e.g., interferon prepared in rabbit kidney works most efficiently in rabbit skin against vaccinia virus). (f) Inhibits multiplication of a large variety of viruses at non-toxic doses. (g) By its mechanism, active virus and cell populations may co-exist over long periods of time (3). (h) Apparently is non-antigenic.

Wagner at Johns Hopkins (4) has observed that reducing the volume of interferon increased the degree of its adsorption and enhances its effectiveness.

III. The Assay for Interferon

A. Chick fibroblast cells are inoculated into sterile Petri dishes. After the cells form a sheet on the bottom of the dishes, the virus is inoculated onto the cells and, after a short incubation period, the infected cell sheet is covered by an agar overlay containing neutral red, a vital dye (7).

B. A small porous cup similar to that used in antibiotic assays is placed on the agar overlay and filled with the test sample of interferon to be assayed.

C. If the sample of interferon inhibits the virus reproduction, the cells around the cup are not destroyed and cytopathogenic effects are not observed. If the interferon has no or little inhibitory activity, the fibroblast cells will be necrotized or destroyed, cytopathogenic effects will be seen, and a clear area or an area filled with cell debris will surround the cup.

D. Some of the factors affecting this assay are, first, the concentration of interferon and, second, the rate of diffusion of interferon through the cup.

IV. The Effect of Tissue Specificity upon Interferon Production

The early work with interferon was done almost exclusively with chick chorio-allantoic membranes, although it was shown that an interferon could be produced in monkey kidney cells (3). Interferons have now been produced in several different host cells, and it appears that, although the products are functionally similar (i.e., they will all inhibit virus growth), they are not identical. This is shown by the cell specificity of the interferons. Interferon prepared in chick cells was much more active when tested in chick cells than when tested in calf cells, and the converse was equally true (3). A similar effect was found with interferons prepared in chick and rabbit cells. This specificity is not always complete, and is not simply an avian-nonavian difference, since interferon prepared in the fertile duck egg is much more active in the duck egg than in the fertile chick egg and vice versa.

The range of viruses that can produce interferon is now quite extensive. It includes such viruses as (1) inactivated influenza types A and B, (2) live influenza virus, (3) Newcastle disease virus, (4) vaccinia virus, (5) avirulent poliomyelitis virus, (6) vesticular stomatitis virus, (7) measles virus, and (8) the encephalitis group of viruses.

The inference to be drawn from these observations is clear. Interferon is no longer viewed as a product of the interaction of one particular virus with one particular cell, but as a general reaction of a wide variety of viruses in a range of different host cells. It is difficult to see what these different viruses have in common, and it may be that interferon is a normal component of the host cell, differing

slightly from cell to cell, but having a similar effect on the host cell-virus interaction (8).

V. The Effect of Nutrition on the Interferon-Virus Relationship

A. Chick embryo cells after growth were kept on a simple (non-nutritional) maintenance medium. They were first inoculated with interferon and then later infected with West Nile virus. This single dose of interferon protected against the virus for the length of the lifetime of the cells. The inhibition was related to cell division which was kept to a minimum by the maintenance medium (9).

B. When the medium was changed every two or three days, so that the excess interferon was removed from the cells at an early stage of the experiment, the cells still showed prolonged resistance to infection with West Nile virus, because the interferon remained in the cells.

C. Fresh nutrients added to the cells encouraged cell division, and virus production increased. It was believed that new cell multiplication diluted out the protective concentration of interferon to a point at which West Nile virus was able to proliferate.

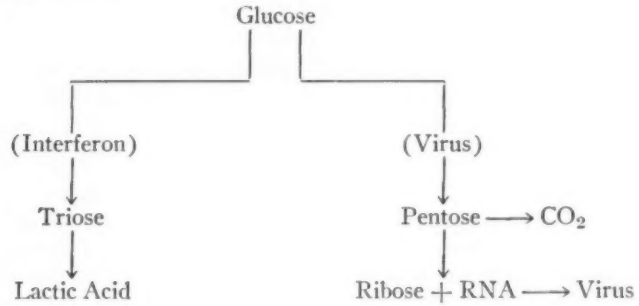
D. The addition of more chick cell interferon again increased cell resistance to the virus. This occurred because new cells produced by the recent cell division no longer possessed interferon, thus the fresh addition of interferon to these cells allowed them to now become resistant to West Nile virus infection.

VI. Mode of Action of Interferon

A. Isaacs observed that tubes of chick embryo fibroblasts exposed to interferon developed two to three times the amount of acid as compared to control tubes inoculated with virus.

B. Cells treated with interferon showed an increased oxygen uptake which was not observed in control or virus tubes, which Isaacs believes is indicative of increased glycosis. It has been observed that critical levels of glucose and NaH_2PO_4 are needed to give effective interferon activity. Galactose and fructose were also able to stimulate interferon effectiveness (9).

C. Finally, Isaacs postulates that interferon may stimulate the utilization of sugar along a metabolic pathway different from that directed by the virus.



Thus, it would appear that interferon follows the triose pathway (Embden-Myerhof), whereas the virus obtains its energy through the pentose system.

D. Both interferon and dinitrophenol appear to exert their activities through similar mechanisms. Both substances: (a) stimulate glycosis, (b) inhibit virus growth in normal cells, and (c) have no virus inhibitor effect in Hela cells (interferon made in Hela cells has been tested in other cell lines such as human amnion).

Isaacs speculates that interferon might stimulate tumor cells because they also get their energy through anaerobiosis and, therefore, it is possible that interferon actually may favor tumor cells and their survival.

VII. Purification of Interferon

Previous studies have shown that interferon is probably a protein. This was inferred from its destruction by proteolytic enzymes, its resistance to ribonuclease and desoxyribonuclease, and its stability characteristics. It was possible to obtain a molecular weight for interferon in the range of approximately 70,000 by means of the diffusion of interferon through an agar gel (8). This is in the range of many proteins, but is too low for a nucleoprotein (10).

Direct evidence for the protein nature of interferon has now been obtained by purification and analysis (10). The crude material was precipitated with ammonium sulfate and chromatographed on modi-

fied cellulose ion-exchange columns. The product obtained gave a single band on starch-gel electrophoresis at two pH values, and gave a single component of $S_{20,W} = 4.77$ when examined in the analytical ultracentrifuge. It is difficult to establish the purity of a macromolecular material, but the results obtained show that the purified preparation is homogenous with respect to charge and size and it is, therefore, justifiable to call it pure (10). The purified material is highly active. When diluted to 6 mg. of protein per ml., it will still, in the test system used, reduce virus yield to 3 per cent of that of the controls.

The purified preparation is a protein with a typical ultra-violet absorption spectrum. Analysis shows that it does not contain nucleic acids and only small amounts of carbohydrate and neuramic acid. Interferons prepared from different host cells will be compared as soon as enough material is available.

Kilbourne *et al.* (11) produced interferon by a method suggested by Wagner. In an interesting experiment, they noted that cortisone "prevented the *in ovo* synthesis of interferon." Furthermore, the action of interferon, already present, was suppressed.

VIII. Possible Uses of Interferon

Interferon can inhibit the multiplication of viruses in tissue-culture (3), in the whole egg (1), and in rabbit skin (15). It is too early to answer the question of what effect interferon might have in the whole animal and in man, although there is some preliminary data which indicates that interferon will confer some protection against certain viruses in mice (12-15). It has been shown that interferon is non-toxic in the doses used so far, and also that it is either non-antigenic or very poorly antigenic. This means that it could be injected repeatedly without any resistance being built up. The range of viruses against which interferon is active is quite broad and now includes poliomyelitis (5) and the encephalitis (4) groups.

Lastly, and most encouraging, interferon has been shown to be involved in the normal host defense mechanism against virus infection. If there is a sufficient amount of the right kind of interferon in the right time, there is a real chance of being able to inhibit virus multiplication and to prevent its damaging consequences.

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COMBINATION CHEMOTHERAPY IN ACUTE LEUKEMIA: MISCELLANEOUS CHEMICALS

By John R. Sampey *

COMBINATION chemotherapy, using two or more drugs simultaneously or sequentially, is being employed in the search for more effective control of malignant blood diseases. Synergistic effects have been observed in previous studies on combination chemotherapy in acute leukemia using folic acid antagonists plus corticosteroids (55), and also in using the combination of 6-mercaptopurine plus steroid hormones (54). A review has been made also of the action of 6-mercaptopurine with a dozen other cytotoxic chemicals in the management of acute leukemia (56). The present study records the results of a search of the original literature for the treatment of acute leukemia with some thirty other combinations of drugs.

Corticosteroids plus Antibiotics. Polli and Eridami (47) induced a number of remissions with the combination corticosteroids plus antibiotics: in eight patients receiving cortisone plus antibiotics, they reported three partial clinical and two partial hematologic remissions; when prednisone was the hormone used in 11 patients, they recorded five complete and four partial clinical responses, and two complete and five partial hematologic remissions; with triamcinolone as the steroid, they noted one complete and two partial clinical remissions, and two partial hematologic remissions in three cases; finally, with desamelasone plus antibiotics, they induced one complete and five partial clinical responses, and four partial hematological remissions in seven patients. Jamra and associates (26) used high doses of prednisone plus antibiotics to secure six complete remissions lasting six months, and seven partial remissions to seven months in 13 patients with acute lymphocytic leukemia; with four cases of acute granulocytic leukemia, they observed one complete remission for four months, and three partial and brief responses. Nicolau *et al.* (43) reported eight considerable and 10 moderate clinical and hematologic improvements in 28 children who were administered the combination cortisone plus antibiotics plus transfusions. Janbon (27) noted a brief response in three of five cases following administration of cor-

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tisone plus actinomycin C. Bovet-DuBois *et al.* (7) used combinations of cortisone and antibiotic effectively in the control of acute leukemia in childhood. Lemoyne *et al.* (33) secured a good hematological remission in one patient after treatment with the antibiotic, oracilline, plus cortisone. A five months' partial clinical and hematologic remission was described by Konchalovskaya (32), after employing the combination ACTH plus antibiotic plus transfusion. Giraud *et al.* (20) used cortisone plus terramycin to secure a brief response in a child with acute granulocytic leukemia; Jonasch (29) recorded the failure of a patient with subacute granulocytic leukemia to respond to the combination cortisone plus antibiotic plus transfusion.

Corticosteroids plus Urethan. Buttaro and Brunori (9) employed prednisone and prednisolone to increase the tolerance to urethan in the management of acute leukemia. Keibl (30) recorded good responses in acute leukemia after use of the combination ACTH plus urethan. Malaguzzi-Valeri (37) described a 2½ months' remission in one patient with acute myeloid leukemia following treatment with cortisone plus ACTH plus urethan. Miranda (41) combined prednisolone, ACTH, urethan, and transfusions to obtain a brief response in one case of acute granulocytic leukemia. The modified corticosteroid, triamcinolone, plus urethan gave symptomatic improvement in one patient with subacute granulocytic leukemia, according to Rosenthal (48). Vignetti (63) induced a remission lasting 320 days in a child with the combination cortisone plus ACTH plus urethan plus transfusions; he reported that another child on urethan and transfusions survived only 48 days, while two on cortisone and transfusions alone survived 195 and 240 days.

Corticosteroids plus TEM. Maggi *et al.* (36) induced a one month remission in a child with the combination prednisone plus triethylene melamine (TEM). Buttaro and Brunori (9) found that prednisone and prednisolone increased the tolerance to TEM of patients with both chronic and acute leukemia.

Combination Corticosteroids. Butzengeiger (10) treated four patients with acute granulocytic leukemia with a combination of hydrocortisone, prednisone, and prednisolone and described four good clinical and hematologic remissions. Two remissions in nine patients with acute lymphoid leukemia were recorded following treatment with cortisone plus ACTH, but no response was found in four patients

with acute myeloid leukemia on the same therapy (61). Malaguzzi-Valeri (37) observed two partial responses in two children with acute leukemia who were given cortisone plus ACTH. Vignetti (63) induced improvement for 188 days in a child who was administered cortisone plus delta cortisone plus transfusions. Schulman (58) noted some palliation in three children after receiving cortisone plus ACTH.

Prednisone plus B. W. 57-323. Selawry and 20 associates (59) in a cooperative study described five partial remissions of bone marrow, and one partial response in all three parameters checked in 13 patients who were administered the combination 2-amino-6-[(1-methyl-4-nitro-5-imidazolyl)thio]purine, or B.W. 57-323, plus prednisone. Rundles *et al.* (50) used the same combination in 13 patients to secure five complete remissions, one good, one fair, and two questionable responses; the complete remissions lasted from two to 10 months.

Corticosteroids plus Radiophosphorus. Koler *et al.* (31) reported statistically significant prolongation of life in 169 patients with acute leukemia who were treated with a combination of corticosteroids plus P-32; patients with subacute leukemia survived twice as long as did those with acute form.

Corticosteroids plus 6-MP plus Antibiotics. Polli and Eridami (47) have reported a number of remissions in acute leukemia following combinated therapy with the three chemicals, steroid hormones plus 6-mercaptopurine (6-MP) plus antibiotics: in six patients who received cortisone plus 6-MP plus antibiotic, they recorded one complete hematologic remission, four partial hematologic, and five partial clinical remissions; in 12 cases on prednisone plus the same two agents, they observed two complete and seven partial clinical responses, and one complete and five partial hematologic remissions; when triamcinolone was the hormone used in two patients, the response was two complete clinical and one partial hematologic remissions. Ooe *et al.* (45) noted little improvement in a patient given prednisone plus 6-MP plus achromycin.

Corticosteroids plus ACTH plus Antibiotics. Polli and Eridami (47) tested two combinations of these three drugs: with cortisone as the corticosteroid, they induced a partial clinical response in one patient and then, with prednisone as the steroid in three cases, they

noted one complete and one partial clinical remission, and one partial hematologic remission on combination chemotherapy.

Prednisone plus ACTH plus E39. These same investigators (47) used prednisone plus ACTH plus ethyleneimino-quinone (E39) in a patient to induce a partial clinical and hematologic response.

Prednisone plus Antibiotics plus Vitamins. Coletta *et al.* (11) secured little response in a child administered the combination prednisone plus antibiotics plus vitamins.

Prednisone plus Myleran plus Methotrexate. Davis and Korst (14) noted no improvement in a patient with subacute granulocytic leukemia and mongolism who received the combination prednisone plus myleran plus methotrexate.

Cortisone plus Testosterone plus Methotrexate. Hansen (21) described remissions to 6½ months in three children who were administered a combination of cortisone plus testosterone derivative, dianabol, plus methotrexate plus x-rays.

Cortisone plus Aminopterin plus Antibiotics. Verrotti (62) employed the combination cortisone plus aminopterin plus antibiotics (penicillin and streptomycin) in the management of two patients. Olmer (44) obtained a complete remission lasting over three years in a patient with undifferentiated acute leukemia who received cortisone plus aminopterin plus antibiotics.

Cortisone plus TEM plus Oligo-Metals. Bovet-DuBois *et al.* (7) preferred the combination of cortisone plus TEM plus Oligo metal catalysts to the administration of the drugs separately in a child.

Prednisone plus Chloronitrin plus Diet. Palitzsch (46) induced a good remission in a child with the combination prednisone plus chloronitrin plus diet.

Cortisone plus ACTH plus Urethan plus Transfusions. Vigetti (63) noted a remission of 320 days in a child following the use of the combination cortisone plus ACTH plus urethan plus transfusions.

Cortisone plus Delta Cortisone plus Amethopterin plus 6-MP. The same investigator (63) also induced a remission in a child for 12 months on the combination cortisone plus delta cortisone plus amethopterin plus 6-MP plus transfusions.

Delta Cortisone plus Urethan plus Colcemid plus TEM plus Mielocin. In a further trial of combination chemotherapy, Vignetti (63) described a good survival for 18 months in a child administered delta cortisone plus urethan plus colcemid plus TEM plus mielocin plus transfusions.

ACTH plus Pencillin plus Vitamins. Danis (13) gave two children a combination of ACTH plus penicillin plus vitamins to induce brief and partial responses.

Folic Acid Antagonists plus Antibiotics. Heinle (24) employed the folic acid antagonists, aminopterin and amethopterin, in combination with penicillin and transfusions to achieve 18 clinical and hematologic remissions for an average of three to four months in 23 children; five showed clinical response only; in eight adults (two with acute lymphocytic and six with acute monocytic leukemia), he observed no response. Aballi and Barreras (1) reported five good hematologic remissions in children administered aminopterin plus antibiotics. Smith and Bell (60) combined aminopterin, penicillin, aureomycin, and transfusions in nine children with lymphoblastic leukemia and described improvement in bone marrow, but they observed no prolongation of life; in acute and chronic myelocytic leukemia, the treatment was without effect.

Folic Acid Antagonist plus TEM. Marmont and Fusco (38) secured some brief remission in one case of acute lymphocytic leukemia, following administration of folic acid antagonist plus TEM.

Folic Acid Antagonist plus CF. Schoenback *et al.* (57) reduced the toxicity of aminopterin and amethopterin in eight patients with acute leukemia by use of citrovorum factor (CF), and they described five partial responses. Jimenez de Asua (28) prevented stomach ulceration with CF in 25 children receiving aminopterin, and he reported 69 per cent remissions which were good though brief. Consoli (12) noted a synergistic effect in the combination aminopterin plus CF in five patients with acute myelocytic leukemia who had good remissions on this therapy.

Folic Acid Antagonist plus TEM plus Nitromin. Aboul-Nasr (2) found no response in four adults who received the combination folic acid antagonist plus TEM plus nitromin.

Folic Acid Antagonist Combination. Wilson (65) induced a remission of several years in a patient with acute monocytic leukemia who was placed on the combination aminopterin plus amino-an-fol.

Folic Acid Antagonist plus FUDR. Hartmann *et al.* (23) observed enhanced toxicity in patients with acute leukemia who were given methotrexate two months before the administration of 5-fluoro-2′-deoxyuridine(FUDR).

Folic Acid Antagonists plus Urethan. Rottini (49) described a good hematologic remission in a child, following combination therapy with teropterin plus urethan. Meyer and Rutzky (40) recorded that amethopterin plus urethan failed to prevent the development of bone lesions in a patient with acute plasma cell leukemia.

Antibiotics plus Urethan. Fornana (18) described remissions in four children with acute lymphoid leukemia after administration of penicillin plus urethan plus transfusions.

Antibiotics plus ACTH or Sodium Nucleate. Konchalovskaya (32) discovered a five months' remission in a patient receiving antibiotics plus ACTH plus transfusions; another patient responded for six months to the combination antibiotics plus sodium nucleate plus transfusions.

Antibiotic plus Vitamins. Lombos (34) induced a complete remission for 8½ months in a patient with subacute lymphocytic leukemia who received penicillin plus vitamin B_6 plus transfusions. In 1950, Barnard and Fox (4) secured an eight months' remission in a patient, following the use of B_{12} concentrate plus terramycin. The next year, Barnard (5) treated 50 patients with acute leukemia with concentrates of B_{12} and antibiotics, and he recorded that the clinical condition was greatly improved but the blood picture remained unchanged.

TEM plus CF. Marmont and Fusco (39) employed CF to reduce granulocytopenia induced in a patient with acute monocytic leukemia who was on TEM therapy.

TEM plus Oligo Metals. DuBois-Ferriere reduced the toxic effects of TEM by the use of metal catalysts of Zn, Cu, and other Oligo metals: in 1951, he described (15) remissions in two patients with acute myeloid leukemia and, two years later (16), he noted that

three patients with acute leukemia responded clinically and hematologically to the same combination but that only one in three subacute had total remission.

N-Mustard plus Urethan plus Antibiotic. Hofstetter (25) reported a remission of 14 months after treatment with the combination nitrogen mustard plus urethan plus penicillin plus transfusions.

N-Mustard plus Cysteine. Weisberger *et al.* (64) discovered that injections of L-cysteine prior to N-mustard therapy permitted larger doses of the cytostatic drug; they described excellent responses in four patients with acute monocytic leukemia, but three were brief.

N-Mustard plus Vitamins. Salamone and Sanfilippo (52) described the disappearance of megaloblasts in the blood of a patient with subacute erythraemic myelosis, following the use of vitamin B_{12} plus N-mustard.

N-Mustard plus Lumin. Nakauchi (42) treated a patient with subacute myelocytic leukemia with the combination nitromin plus the cyanine dye, lumin; a hematologic remission was achieved.

N-Mustard plus Plasma Proteins. Brennan *et al.* (8) made use of proteinamine, a combination of N-mustard and plasma proteins to secure two clinical responses in seven cases of acute lymphocytic leukemia; one case of acute myelocytic leukemia did not respond.

Vitamin C plus Toluidine Blue plus Hesperidin Methylchalcone. Gay and Montale (19) had five good responses in 10 patients with acute leukemia, following the use of the combination vitamin C plus toluidine blue plus hesperidin methylchalcone.

Discussion

Corticosteroids dominate this review, for they have been employed in one-half of the two score combinations of cytostatic drugs cited. However, only the combination of steroid hormones plus antibiotics have been tested sufficiently to evaluate the results: tabulations on the reports which give both the number of patients treated and the number responding show that corticosteroids plus antibiotics gave a remission rate of 80 per cent in four score patients. Combination chemotherapy with these two most frequently used cytostatic drugs

in acute leukemia should be given more extensive clinical trials. Urethan and N-mustards are two other drugs which have attracted considerable attention in combination chemotherapy, but the number of patients is too limited for evaluation. Fear of increased toxicity from the use of two or more cytostatic drugs is not reflected in the reports of this study from the original literature.

Acknowledgments

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SELECTED ABSTRACTS

A Suppository Substitute for an Enema. Feder, I. A., *et al. Am. J. Gastroenterol. 33:366* (1960). Fecal impaction is a common condition encountered among hospitalized patients, particularly those that are aged and debilitated. The usual oil or soap enemas are often resented and ineffective; they may cause tenesmus or intestinal colic; and they require much nursing time. Consequently, the authors evaluated a suppository that releases carbon dioxide in the rectum, as a substitute for an enema. The release of an adequate amount of this gas distends the rectum and induces a normal defecation reflex. The accumulation of feces in the rectum has resulted from the impairment of the defecation reflex in constipation. Constipation, in turn, resulted from inactivity, restricted diets, the use of constipating drugs, fear of pain, etc.

The suppositories employed contained the following: sodium bicarbonate 0.6 Gm., potassium bitartrate 0.9 Gm., calcium silicate 0.05 Gm., bentonite 0.3 Gm., polyethylene glycol 0.2 Gm., dextran (282) 0.05 Gm., cocoa butter 0.05 Gm., vegetable lecithin 0.1 Gm., and talc q.s. If no results were obtained in one-half hour after the insertion of the suppository, a second suppository was inserted. Most patients were found to need two suppositories.

Of 150 medical patients given the suppositories, 90 had adequate evacuations but inadequate results were obtained in the other 60 patients. Of 50 postoperative patients, 33 had adequate and 17 had inadequate evacuations. In another group of 25 patients being prepared for proctosigmoidoscopy, the insertion of two suppositories produced evacuations satisfactory for adequate observation in 14 of the patients. Therefore, the authors concluded that these suppositories were worthy of further evaluation as a satisfactory and time-saving substitute for enemas in hospitalized patients afflicted with constipation.

Gelatin Capsules for Rectal Administration. Wellauer, J., del Buono, M., Ory, A. M., and Steiger-Trippi, K. *Pharm. Acta Helvet. 35*:619 (1960). The administration of drugs by means of elastic gelatin capsules, about 1 inch in length, was recommended by the authors as a new form for rectal administration and one from which the drug would be released more quickly. The release of the medication, barium sulfate in the studies reported, was evaluated by means of the Erweka tester, a distintegration time tester, and by clinical observation by means of x-rays.

The gelatin capsules were somewhat tear shaped and were filled with an oily suspension containing 800 mg. of barium sulfate. The controls were molded suppositories, each containing 800 mg. of barium sulfate. One form of the suppositories was made from a base of adeps neutralis, a controlled combination of glycerides of fatty acids. The other form of suppositories was made from a combination of polyethylene glycols; namely, polyethylene glycol 6000—47 parts, polyethylene glycol 1540—33 parts, and polyethylene glycol 400—20 parts.

It was found that the gelatin capsules began to disintegrate in the rectum in an average of less than 8 minutes, many capsules having begun to disintegrate in less than five minutes. The polyethylene glycol suppositories began to disintegrate in about 10 minutes and the adeps neutralis suppositories in an average of about 15 minutes. The gelatin capsules could be easily inserted by first wetting them slightly in water. After insertion the capsules soften and then the pressures of body movement and peristalsis cause the capsules to burst.

The authors also pointed out that the gelatin capsules could be stored at a higher temperature than the suppositories.

BOOK NOTICE AND REVIEW

Grundstoffe und Verfahren der Arzneibereitung. Prof. Dr. Fritz Gstirner. 1006 pp., 116 illustrations, and 154 tables. Publisher: Ferdinand Enke, Stuttgart, West Germany. Price: paperbound, about $33.50; linenbound, about $35.

This text deals with the methods of preparing dosage forms. Special emphasis is placed on vehicles or carriers which are necessary to prepare medication which is not only acceptable to the patient, but will also produce stable and potent products.

The following dosage forms are discussed: tablets, injectables, suppositories, pills, emulsions, syrups, ointments, powders, liniments, suspensions, and ophthalmic solutions. The author based his discussions on German, English, American, French, and Danish publications. He does not give a critical appraisal of the different methods discussed, but leaves it to the reader to select a specific method for his own compounding or manufacturing problem. A great number of manufacturing formulas are listed with properties of diluents or carriers to be used.

This text is an excellent reference book which should be on the shelf of every pharmacist in industry and in general practice.

ELSA EHRENSTEIN

British National Formulary, 1960, Standard edition. British Medical Associates and the Pharmaceutical Society of Great Britain. 272 pp. The Pharmaceutical Press, 17 Bloomsbury Square, London, W. C. 1. Price: 70 bd.

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